

Illustration for Supplementary datasets

Supplementary dataset 1: List of 14006 non repetitive novel piRNAs predicted from both pseudo-males (ZW) and male (ZZ) donors of *Cynoglossus semilaevis*.

Supplementary dataset 2: List of 7070 novel piRNAs categories in ZZ donor group.

Supplementary dataset 3: List of 11588 novel piRNAs categories in ZW donor group.

Supplementary dataset 4: List of 26135 differentially expressed piRNAs identified according to the criteria detailed in the methods section in the males and pseudo-males.

Supplementary dataset 5: List of Target genes of the 44 candidate signature piRNAs that differentially expressed in males and pseudo males.

Supplementary dataset 6: List of 37 candidate signature piRNAs captured from 44 candidate signature after sex related gene ontology (GO) enrichment and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment analysis.

Supplementary dataset 7: The expression profiles of 37 signature piRNAs by small RNA sequencing in males and pseudo males

Supplementary dataset 8: List of 53 DNA methylation related target genes identified that predicted to interact with 15 regulating piRNAs.

Supplementary dataset 9: List of 16 target genes related to transposition, especially heterochromatin formation identified together with their 10 interacting piRNAs.

Supplementary dataset 10: The RT-qPCR data of the expressions of all six piRNA markers

Supplementary dataset 11: List of piR primers of six piRNAs in the RT-qPCR.